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Assessing past water availability using phytoliths from the C_4 plant *Sorghum bicolor*: an experimental approach

Keywords: Phytoliths; Sorghum bicolor; C_4 plants; Irrigation; Water Availability; Jordan

Highlights:

- Sensitive:fixed phytolith ratios in sorghum were affected by water availability
- Sensitive:fixed phytolith ratios were greatest in sorghum husks
- Sensitive:fixed phytolith ratios in sorghum were higher than in C_3 plant husks

Abstract

Water availability and water management systems were critical for the success of past agricultural societies. One way to determine past water availability is through phytolith ratios

as demonstrated by research conducted on modern C_3 plants. In order to determine if phytolith ratios in C_4 plants are similarly affected by plant water availability, the C_4 plant *Sorghum bicolor* was experimentally grown at three different crop growing stations over a two year period in Jordan. The husks, leaves and stems of the plants grown under the 0% and 100% irrigation regimes were processed and analysed for their sensitive to fixed form phytolith ratio. These results were then compared to results of those conducted using C_3 plants. Our results showed that while there were differences in ratios between growing years and the crop growing site, the greatest difference in the ratios was in irrigation regime. Our results also showed, however, that the ratio of sensitive to fixed forms for the samples taken from the husks was far higher than the ratios found in the leaves and stems and far higher than those found in previous studies on C_3 grasses. We suggest that if this method is to be used to interpret archaeological phytolith assemblages, an assessment of the likely taxa and plant part composition of the assemblage should first be undertaken through phytolith and macro-botanical analysis.

1. Introduction

Water availability and water management systems were critical for the success of early agriculture and the development of complex societies. Crop failure due to insufficient rainfall and flooding (both too little and too much) would have been a problem in many global regions and as such some form of irrigation and water management, including drainage systems, must have been employed to safeguard against this. These water management features are, however, difficult if not impossible to recognise in the archaeological record, particularly from prehistoric periods, when many of these would have been ephemeral and as such would not have survived. Even the larger more elaborate features such as ridged fields and dams may no longer be visible in the archaeological record due to their erosion through time. This leaves a critical lack of understanding in our knowledge of how our ancestors managed to successfully transition from hunter-gatherers to farmers and, in later times sustain large populations in complex societies, often against the backdrop of challenging climates and environmental conditions (see Mithen 2012 for a discussion of past water management).

One of the most effective ways to determine if water management systems were used to grow crops is by looking directly at the botanical remains themselves. This way if other environmental proxies suggest that environmental conditions were arid or semi-arid during the period of occupation, but the botanical remains indicate that the crops received adequate amounts of water, then it is possible to infer deliberate water management rather than favourable environmental conditions.

It was initially suggested that grain size could be used to assess past crop water availability. Helbaeck (1960) used the size of charred flax seeds to determine how much water the flax received during growth while Mabry et al. (1996) used wheat grains from Tell Handaquq to infer irrigation. This method can be problematic, however, because taphonomic processes such as charring can distort the size and shape of seeds (Märkle and Rösch 2008). Another method which uses macro-botanical remains directly is the FIBS (Functional Interpretation of Botanical Surveys) approach. This relies on the analysis of the accompanying crop weeds to infer past water availability (e.g. Jones et al. 1995; Charles et al. 2003). Another more recent approach which also relies on macro-botanical remains is the use of carbon isotope stable discrimination (Δ 13C). This method was pioneered by Araus et al. (Araus and Buxó 1993; Araus at al 1997; Araus et al. 2003; Araus et al. 2007) and has been used effectively by other researchers-for example: Caracuta et al. (2015); Ferrio et al. (2005), Fiorentino et al. (2008); Masi et al. (2014); Mora-González et al. (2018); Riehl et al. (2008); Roberts et al. (2011); Flohr et al. (2019). While looking directly at the macro-botanical remains from archaeological sites may be the most direct to establish how much water the crop received during growth, macro-botanical remains require specific preservation conditions in the archaeological record (e.g charring, water-logging, desiccation) which does not always occur. As a result, macro-botanical assemblages can often be scarce on archaeological sites, particularly on early archaeological sites or in sites with unfavourable preservation conditions.

One method for identifying the level of past crop water availability that does not rely on the preservation of macro-botanical remains is the phytolith water availability index (Madella et al. 2009; Jenkins et al. 2011; Weisskopf et al. 2015; Jenkins et al. 2016). Phytoliths are microscopic structures which are mainly composed of silicon dioxide, also known as silica (SiO_2), which is absorbed by the plant in a soluble state from the ground water during transpiration (Piperno 2006). In solution, silica usually exists as monosilicic acid (H_4SiO_4) which

is transported upwards through the vascular system of the plant. This then gels, solidifies and forms into solid opaline silica (SiO_2:nH_2O) bodies (phytoliths) which form in the cell wall, the cell lumen and the intercellular spaces (Sangster et al. 2001).Silicon (Si) uptake and deposition in plants is not well understood with the first Si transporters being identified in rice (*Oryza sativa*) which is a hyper-Si accumulator (Ma 2006). Currently, three main different types of Si transporters are known in rice: Low Silicon 1 (LSi1) which allows the passive transport of Si across the plasma membrane between the apoplast and the plant cells; Low Silicon 2 (LSi2) which facilitates the active transport of Si out of the plant cells (Ma 2006; Tubuna and Heckman, 2015); and Si transporter (Lsi6) which is responsible for xylem unloading (Yamaji et al. 2008). Research is on-going to identify similar Si transporters in other Graminae species for example barley (*Hordeum vulgare*) (Chiba et al. 2009; Yamaji et al. 2012); maize (*Zea mays*) (Mitani et al. 2009); wheat (*Triticum aestivum*) (Montpetit et al. 2012); and sorghum (*Sorghum bicolor*) (Markovich et al. 2015).

Because they are made of silica, phytoliths are inorganic and preserve well in the archaeological record. They provide useful information about paleoecology (e.g. Coe et al. 2014), past diet, ritual practices (e.g. Power et al. 2014), craft activities (e.g. Wendrich and Ryan 2013) and, as stated above, past water availability in plants (Rosen and Weiner 1994; Madella 2009; Jenkins et al. 2011; 2016; Weisskopf 2015).

This latter approach of using phytoliths as an indicator of past water availability was initially proposed by Rosen and Weiner (1994) who found through an experimental study that the number of conjoined cells in multi-celled phytoliths from emmer wheat (*Triticum turgidum* subsp. *dicoccum*) and bread wheat (*T. aestivum*) grown in arid and semi-arid conditions increased with water availability. While this study was pioneering, it was subsequently discovered that taphonomic processes can break down multi-celled phytoliths and reduce the number of conjoined forms thereby questioning the efficacy of this method for phytolith assemblages with unknown taphonomic histories (Jenkins 2009; Shillito 2011a).

An alternative method for assessing past water availability using phytoliths was later explored with promising results (Madella et al. 2009; Jenkins et al. 2011; Weisskopf et al. 2015). This method involved grouping phytoliths from grasses into forms whose production is genetically

controlled, known as *fixed* forms and into forms whose production is environmentally controlled known as *sensitive* forms (Madella et al. 2009; Jenkins et al. 2011; Weisskopf et al. 2015). Fixed forms include the short cells such as rondels, saddles, bilobates and crosses and because silica deposition in these cells is genetically controlled they will form phytoliths regardless of how much water is available in the growing medium. In contrast, are the sensitive forms which comprise mainly long cells and the stomata; their silicification is governed by environmental variables a major one being water availability (Blackman and Parry 1968, 1969; Kaufman et al. 1970, 1981; Piperno 2006).

Madella et al. (2009) first suggested using phytolith ratios as a method for identifying past water availability. In this study, five cereals were grown under two different climatic regimes: Middle Eastern and Northern European. Middle Eastern conditions were simulated using a growing chamber with controlled light, heat and day length while the Northern European climatic conditions were represented by open fields in Cambridge (Madella et al. 2009). The cereals used in this experiment were: bread wheat (*T. aestivum*), emmer wheat (*T. dicoccum*), spelt wheat (*T. spelta*), two row barley (*H. vulgare*) and six row barley (*H. distichon*). These were grown under two different irrigation regimes wet and dry. Phytoliths from the leaves of all plants were analysed, while phytoliths from the stems were also analysed for emmer and spelt wheat (Madella et al. 2009). The results showed that there was a slight increase in the ratio of sensitive to fixed forms under the wet regime compared to the dry regime in the samples studied with the exception of the leaves of the six-row barley (Madella et al. 2009; see Table 1 this study for the means of the sensitive to fixed forms from Madella et al. 2009).

Another experimental study involved growing native land races of durum wheat (*T. durum*) and six-row barley (*H. vulgare*) in three different crop growing sites in Jordan for a three year period. The crops were subjected to different irrigation regimes: (1) no irrigation- 0% of crop water requirements (CWR); (2) under irrigated – 80% of CWR; (3) irrigated – 100% of CWR; and (4) over irrigated – 120% of CWR; a fifth regime, 40% of CWR, was added in the second and third growing season (Jenkins et al. 2011; 2016). Rainfall, pan-evaporation, soil conditions, and plant available Si were monitored and phytoliths from the husks (inflorescence bracts comprising glumes, lemmas and paleas) were analysed for sensitive to fixed phytolith ratios. These results showed that that when rainfall was between 100 millimetres and 250

millimetres per annum a ratio of sensitive to fixed forms of >1 meant that irrigation could be predicted with 80% confidence. When rainfall was less than 100mm, the explanatory power was greater with a ratio of >0.5 meaning, irrigation could be predicted from the phytolith ratios with 99% confidence (Jenkins et al. 2016).

Weisskopf et al. (2015) used this method to identify the growing conditions of rice during the Neolithic in the Lower Yangtze valley, China. They took soils samples for phytolith analysis from modern rice fields in India which comprised the phytoliths not just from the rice but also from the accompanying arable weeds. These fields were categorised according to water availability as: 1) dry, rain-fed, and margin of wetland; 2) very wet, in standing water throughout most of the growing season; or 3) intermediate (Weisskopf et al. 2015). They found that the percent of sensitive to fixed forms could be used to monitor wetter and drier growing conditions in the modern assemblages. They then applied this method to archaeological sites in China and were able to identify changes in rice cultivation from flooded and drained fields to intensively irrigated paddies (Weisskopf et al. 2015).

While these studies highlight the efficacy of using the phytolith water availability index on assemblages from C_3 plants- wheat, barley and rice, aside from any C_4 arable weeds that may have become incorporated into the soil samples from the fields analysed by Weisskopf et al. (2015), there has been no analysis conducted on C_4 plants directly. Generally, plants can be classified according to their carbon fixation pathway. The C_3, Calvin-Benson, pathway is so-called because the first molecule produced in the cycle is a 3-carbon molecule. This pathway is typical of temperate species including most trees and shrubs, many fruits and vegetables and a large proportion of the cereals of economic importance such as wheat, barley, oat, rye and rice (Boutton 1991; Sage et al.1999; 1999, Sealy 2001). The C_4, or Hatch-Slack, photosynthetic pathway is so –called because the first step of photosynthesis in this pathway involves fixation of Carbon dioxide (CO_2) in the mesophyll cells to form a 4-carbon acid. This pathway is mainly comprised of plants adapted to warm and (semi-) arid environments (Boutton 1991a) although they are also found in wet conditions provided that there is ample warmth and light. C_4 plants which are of economic importance include maize, millet and sorghum (Sage et al. 1999).

It is unclear how results from the application of this index to C_4 plants would compare to results obtained from C_3 plants. This is unfortunate because, as stated above, there are C_4 species of economic importance and, moreover, archaeobotanical assemblages can be comprised of mixed C_3/C_4 plant assemblages so assessing the validity of this approach in C_4 plants is vital if this method is to be applied to archaeological assemblages with confidence.

In order to address this problem, we conducted a two year crop growing experiment using *Sorghum bicolor* (sorghum) to determine if phytoliths from a C_4 plant can be used to assess past water availability and how these results compare to those from C_3 plants. *S. bicolor* is in the subfamily Panicoideae of the Poaceae family, and is believed to have been domesticated in Eastern Sudan around 3000 BC (Beldados et al. 2015; Fuller and Stevens 2018). From here it spread to south Asia at around 2000 BC and to the Niger Basin in West Africa sometime after 1000 BC. Along with pearl millet, sorghum is one of the two main cereal crops, and the most productive rain fed cereal crop, to originate in Africa (Fuller and Stevens 2018). As such this species was chosen for this experiment due to its great economic importance.

2. Materials and method

2.1 Crop Growing

S. bicolor was experimentally grown as part of the University of Reading's *Water, Life, and Civilisation project* in collaboration with NCARE (National Centre for Agricultural Research and Extension, Jordan). This crop growing experiment was a follow on project to the experimental growing of wheat and barley reported in Jenkins et al. (2011, 2016). The sorghum was purchased at an Amman market and was grown at three different crop growing stations, two of which were the same as those used in the previous study: Deir 'Alla (DA) which is in the Jordan valley and is 200 m below sea-level and Ramtha (RA) which is located in the north of Jordan on the Jordanian plateau at an altitude of 510 m. The third site used was a farm near Salt (SF) which is also located on the Jordanian plateau at an altitude of c 820 m (Figure 1). This latter site was used instead of Kherbet as-Samra which was the third crop-growing site included in the study reported in Jenkins et al., (2011, 2016). This was because Kherbet as-Samra was being discontinued as an NCARE crop growing site at this time.

S. bicolor was grown over two years from 2009 to 2010 and was sown in April and harvested in September/October. In the first year there were not enough grains and so new seeds had to be acquired the following year which appeared to belong to a different, taller variety of *S. bicolor* (Flohr, 2012; Flohr et al. 2019). The crops were grown in 5 x 5 m plots, with 1.5 m in between each plot. Different irrigation regimes were employed: (1) no irrigation-0% of Crop Water Requirements (CWR); (2) under-irrigated-80% of CWR; (3) irrigated-100% of CWR; (4) over-irrigated-120% of CWR. The calculation for irrigation levels was based on knowledge of crop water requirements estimated by using Class A – Pan Evaporation readings (Allen et al. 1998).

Water was implemented by a drip irrigation system with a 60 cm spacing between water pipes and a 40 cm spacing between the drippers on each pipe (Jenkins et al. 2011). Each irrigation plot had eight lines. The water used for irrigation was treated wastewater at Ramtha, a mixture of treated wastewater and fresh water at Deir 'Alla and fresh water at Salt. No additional fertilisers or pesticides were applied. There was no rainfall at any of the sites over the three years because the crops were grown over the summer months. Crops were harvested in 50 cm intervals diagonally across the plot from the outside corner to the middle of the plot at 0-50 cm, 50-100 cm, 100-150 cm, 150-200 cm, 200-250 cm and 250-300 cm spacings. This was done to avoid edge effect (where plants on the edge of plots receive more water and nutrients from the ground because of the decreased competition from other plants). After harvesting, the crops were stored in paper bags and exported to the UK for analysis.

2.2 Phytolith Processing and Counting

All phytolith processing was undertaken at Bournemouth University, UK. Plants from the 0% and 100% irrigated plots only were analysed with the other irrigation regimes being omitted. This decision was made because it was deemed to be more beneficial to analyse all plant parts (husks, leaves and stems) than to include the 80% and 120% irrigation samples and time conöaints did not allow us to do both. Where possible, 15 samples were taken from across each of the different plots (each year, site and irrigation regime): five from the husks, five from the stems and five from the leaves. While all of the 100% irrigated samples had husks

only the 0% irrigated plants from Deir ‘Alla had developed inflorescences and therefore only the husks from ‘Deir Alla were analysed. Phytolith extraction followed the dry ashing method (Table 2) and weight percent of phytoliths was calculated by expressing the weight of phytoliths to original plant matter processed (phytolith weight % = weight of phytoliths/weight of plant matter processed x 100).

Slides were counted using a Meiji MT4300 Infinity polarising microscope at x400 magnification with an attached Canon camera. Phytoliths were grouped according to the mechanism of silification in their production:

- *Fixed forms* (short cells): silification is under genetic control (saddle, bilobate, polylobate, cross)
- *Sensitive forms* (long cells and stomata cells): silification is under environmental control (elongate smooth, elongate sinuate, elongate dentate, elongate dendritic, stomata) (Madella et al. 2009; Weisskopf et al. 2015).

Four hundred phytoliths were counted per slide equalling a total of 57,200 phytoliths. Figure 2 shows images of the phytolith forms found in this analysis.

2.3 Statistical Analyses

All statistical analyses was conducted using R. The sensitive to fixed phytolith ratio was calculated and a log10 transformation was used as a dependent variable in the analysis. The log10 transformation allowed the data to meet the assumptions of parametric statistics through examination of residual and normality plots as per Zuur et al. (2009). A three-way ANOVA design with all interactions was conducted using 'Year', 'Site' and 'Irrigation' as fixed factors in the analysis. Tukey tests were also employed to find out which site means (Deir ‘Alla, Ramtha, Salt) (compared with each other) were significantly different.

3. Results

Our results show that there were significant differences in the mean ratio of sensitive to fixed phytolith forms between crop growing years (explaining 2.73% of variability in the data) and between crop growing sites (explaining 5.22% of variability in the data - Figure 3; Table 3). Tukey tests indicated that Salt is significantly different to Deir 'Alla (p<.0.05), that Ramtha is borderline significantly different to Deir 'Alla (p=0.072) and that Ramtha is not necessarily different to Salt. Our results also show, however, that these differences are far less important than the difference between the irrigated and unirrigated plants (explaining 34.95% of variability in the data - Figure 4; Table 3). Figure 4a compares the mean of the sensitive to fixed forms for the unirrigated and irrigated samples for all plant parts combined. From this it is apparent that the irrigated samples have a much higher mean ratio (4.5) than the unirrigated samples (0.6) but it also clear that when the data are analysed by individual plant part; i.e. husk, leaf and stem, that the husks have a much higher ratio of sensitive to fixed forms for both the irrigated and the unirrigated samples than the leaves and stems (Figure 4b-d). This is in-line with the results for the weight percent of phytoliths to original plant matter which demonstrates that in both 2009 and 2010 the weight percent for the husks was higher than for the leaves and stems suggesting that the uptake of silica into the husks is greater than in the leaves and stems (Figure 5A and B). When this, however, is compared to the *T. durum* which was experimentally grown at Kherbet as Samra from 2004 to 2005 as part of the experiments outlined in Jenkins et al. (2011; 2016) we see that the results are not in accord (Figure 4C). From this figure it is clear that for wheat it is the leaves that have the highest weight percent not husks. Overall, our results show that *S. bicolor* phytolith assemblages with a mean ratio of sensitive to fixed forms >1 are likely to have been irrigated while those with ratios <0.5 are unlikely to have been irrigated. Our results, however, also show that the plant part in which the phytoliths formed is critical and greatly affects results.

Table 1 compares these results to results from previous studies of phytolith ratios in irrigated and unirrigated plants (Madella et al. 2009; Jenkins et al. 2011, 2016; Weisskopf et al. 2015). From this it is clear that the ratio of fixed to sensitive forms for *S. bicolor* is higher than the ratios found in the husks for *H. vulgare* and *T. durum* for both the irrigated and unirrigated plants. In fact, the ratio of sensitive to fixed forms from the husks of the irrigated *H. vulgare* (3.33) is comparable to the ratio found in the husks from the unirrigated *S.*

bicolor (3.86). It also demonstrates that there is much variability in the range of ratios with the ratio of sensitive to fixed forms from the husks of the unirrigated *T. durum* (1.25) being similar to the irrigated ratio from the leaves of *S. bicolor* (1.37) (Table 1). Furthermore, it is apparent from this table that while the results from the *S. bicolor*, those taken from Jenkins et al. (2016) and in Weisskopf et al. (2015) are all broadly comparable the results reported in the Madella et al. (2009) study are, with the exception of the *H. distichon* leaf, lower than those found in the other studies (Table 1).

4. Discussion

Our results found that while 'site' and 'year' had some effect on phytolith ratios 'irrigation' was the variable with by far the strongest explanatory power, indicating that water availability is the most important factor in determining the ratio of sensitive to fixed forms in *S. bicolor*. It is not clear what causes the inter-site differences observed but it is likely due to an interaction of different environmental factors. All three sites have different micro-climates, soil chemistry, soil and water salinity levels, humidity levels, and evaporation rates, all of which could have affected water uptake and phytolith production. The inter-play of such variables is difficult to untangle in the experimentally grown crops as demonstrated in earlier studies (Jenkins et al. 2011, 2016) and would be impossible to assess in archaeological assemblages.

Similarly, the inter-annual variation in the mean between the two growing years could have been caused by a combination of genetic and environmental factors. Two different varieties of *S. bicolor* were sown in the two years, with the one in 2010 belonging to a taller variety than the one in 2009. The 2009 variety could have been more easily affected by differences in temperature between the sites or the 2010 variety could have grown larger roots, thereby accessing water from neighbouring plots, leading to less water stress than experienced by the 2009 variety (Flohr 2012).

The higher proportion of sensitive forms in the husks is interesting and could partly be related to Si uptake into the sorghum husks as suggested by the greater weight percent of phytoliths found in the husks (Figure 4). This is in accord with what is currently understood about Si

uptake in the husks of some species. Hutton and Norrish (1974) found that Si is concentrated in the husks in wheat and that they contain about one-third of the total Si in the plant while Handreck and Jones (1968) found that 40% of the Si weight of the wheat they studied is found in the husks. Similarly, Handreck and Jones (1968) showed that, of all plant parts in oat, the husks contained the highest concentration of Si. In rice and barley, it has been suggested that Si is deposited in the husks at the reproductive stage and that a high accumulation of Si in the husk is important for grain fertility. It has been proposed that this is because Si decreases water loss and prevents against pathogens and that Si may be actively redirected to the husks by Si-mediated transporters (Ma and Yamaji 2014). Analysis of wheat grown as part of a previous crop growing experiment that we conducted in Jordan found contradictory results (Jenkins et al. 2011 and 2016). In this experiment we found that in *T. durum* the highest weight percent of phytolith to original plant matter was in the leaves (Figure 4).

Previous research suggests that sorghum, and Panicoideae grasses (a C_4 sub-family), in general are more prolific producers of elongate dendritics (sensitive forms) than the C_3 grasses (Novello and Barboni (2015). Novello and Barboni (2015) conducted a study evaluating how effective various different African grass species are at producing elongate dendritics. They processed 67 African grass species and their results from the husks of the plants showed that *Sorghum purpureo-sericeum* was the second most prolific producer of elongate dendritics with 52% of the assemblage comprising this form. The most prolific producer, with 77% elongate dendritics, was also a Panicoideae grass- *Sorghastrum stipoides*. In contrast, these species was found to produce few rondels (a fixed form) with *c*5% or less of the assemblage consisting of this form (estimated from Novello and Barboni 2015, Figure 4). In contrast Albert et al. (2008) found that in domesticated wheat, barley and oat, all C_3 grasses, elongate dendritics comprised 7 to 8% of all morphotypes present.

The large range in ratios of sensitive to fixed forms inevitably has an impact on the use of this method to identify past plant water availability in archaeological assemblages. There are, however, ways to mitigate the extent of this impact. For some sites and regions one can be geographically and temporally informed of the plants likely to be available. For example if one were analysing a Neolithic southwest Asian site one would not expect to find sorghum which was likely introduced to this region in the Islamic period based on written evidence (Decker, 2009; Watson 1983). Analysis of the phytolith assemblage and ideally the macro-botanical

353 assemblage, can provide information about the different taxa in the assemblage and the
354 ratios in which they are found which can be used to conduct informed analysis using the
355 method outlined here. Similarly, the phytolith assemblage could be analysed to determine if
356 all plant parts are present or if crop processing was likely to have taken place off site resulting
357 in a higher proportion of husks compared to leaves and stems (Harvey and Fuller 2005).
358 Furthermore, information about the micro-context and taphonomic processes that have
359 affected the phytolith assemblage and potentially impacted the phytolith record can be
360 obtained from targeted micromorphological analysis of the archaeological contexts from
361 which the phytolith samples were taken (Cabanes et al. 2011; Shillito 2011b). These analyses
362 could then be used to inform interpretation of the ratios of sensitive to fixed forms. In general,
363 it is clear from this and previous studies that the ratio of sensitive to fixed phytolith form
364 ratios does increase with increased water availability and further work is now needed to
365 determine how the ratios of sensitive to fixed forms from other C_4 plant species, particularly
366 in the husks, are affected by water availability.

367

368 **5. Conclusion**

369 This study focused on establishing whether the ratio of sensitive to fixed phytolith forms in a
370 C_4 grass, *S. bicolor*, was affected by plant water availability as has been found to be the case
371 in C_3 grasses (Madella et al. 2009, Jenkins et al. 2011, 2016 and Weisskopf et al. 2015). Our
372 results showed that while there were differences in ratios between crop growing years
373 (explaining 2.73% of variability in the data) and between crop growing sites (explaining 5.22%
374 of variability in the data) the greatest difference was found between the irrigated and
375 unirrigated plants (explaining 34.95% of variability in the data). Results also showed that the
376 difference in the ratios between different parts of the plant- husks, leaves and stems –was
377 great with husks having a far higher ratio of sensitive to fixed forms than the leaves and stems
378 (Figure 2). Furthermore, when these ratios are compared with those from the C_3 grasses
379 included in previous studies, the ratio for the husks was found to be much higher. This has
380 implications for using this method to establish past plant water availability on an
381 archaeological site comprised of a mixed C_3/C_4 species assemblage. This is because the
382 average ratio would be calculated from both the C_3 and the C_4 plants and as such would

represent neither pathway (the C_4 plant ratio would be diluted by the C_3 plant ratio and the C_3 plant ratio would be elevated by the C_4 plant ratio). It is suggested that this method is used in an informed manner to interpret past water availability by establishing the likely species and plant part composition of an archaeobotanical assemblage through analysis of its phytolith, and where available, its macro-botanical assemblage. Further work is now needed to establish if this higher husk ratio is consistent in other C_4 species.

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580 **Captions**

581 Table 1 Mean ratio of sensitive to fixed phytolith forms for studies focused on the
582 development of a phytolith water availability index. Results show means from this research
583 focused on *Sorghum bicolor*; Jenkins et al. 2016 focused on *Hordeum vulgare* and *Triticum*
584 *durum*; Weisskopf et al. 2015 focused on *Oryza sativa* [data generated by Weisskop and
585 kindly provided by Fuller]; and Madella et al. 2009 focused on *H. vulgare*, *H. distichon*, *T.*
586 *aestivum*; *T. dicoccum*, and *T. spelta*

587 Table 2 Dry ashing method used to extract the phytoliths from the experimentally grown
588 *Sorghum bicolor*

589 Table 3 Three way ANOVA of the log10 of sensitive to fixed phytolith ratio at different sites,
590 irrigation levels and years

591

592 Figure 1 Map showing the locations of the crop growing sites (triangles). The sites included
593 in this study were Deir 'Alla, Ramtha and Salt while the site of Khirbet as-Samra was used
594 instead of Salt in the study reported in Jenkins et al. (2001, 2016).

595 Figure 2 Photomicrographs of phytoliths from the experimentally grown *Sorghum bicolor*: A)
596 rondels (fixed form) from Ramtha 100% irrigated 2009 husk; B) Bilobates (fixed form) from
597 Salt 100% irrigated stem; C) Bilobates (fixed forms) interspersed with elongate dentates
598 (sensitive form) from Deir Alla 100% irrigated stem D) Elongate dentates (sensitive form)
599 interspersed with rondels from Ramtha 2009 100% irrigated stem; E) Elongate dentates

(sensitive form) interspersed with rondels (fixed form) from Deir Alla 2009 100% irrigated husks; F) Bilobates (fixed forms) interspersed with elongate dentates (sensitive form) from Ramtha 2009 100% irrigated stem; G) Bilobates (fixed form) interspersed with elongate dentates (sensitive form) Ramtha 2009 100% irrigated stem

Figure 3 Mean ratio of sensitive to fixed phytolith forms from the experimentally grown *Sorghum bicolor* with 95% confidence interval A) mean ratio by growing year; B mean ratio by crop growing site

Figure 4 Mean ratio of sensitive to fixed phytolith forms with 95% confidence interval for samples from the unirrigated compared to irrigated *Sorghum bicolor*: A) all plant parts; B) husk; C) leaf; and D) stem

Figure 5 weight % of phytolith extracted to original plant matter processed by plant part: A) *Sorghum bicolor* (2009); B) *Sorghum bicolor* 2010 and C) *Triticum durum* based on 20 plants grown as part of the experiments reported in Jenkins et al. (2011, 2016).